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FACTORS AFFECTING THE DEVELOPMENT OF RESISTANCE TO TRAUMATIC SHOCK IN THE RAT¹

BY C. GWENDOLINE TOBY² AND R. L. NOBLE³

Abstract

Experiments have been conducted on rats to determine what factors may influence mortality and resistance, which have previously been shown to follow traumatic shock produced by the drum method. The resistant condition followed a single exposure to trauma and developed after a latent period of 24 hr. It lasted for four to six days. The degree of resistance was proportional to the severity of the initial trauma.

The percentage mortality following continuous and interrupted trauma was determined. Rest intervals between each 100 turns of the drum allowed greater tolerance to trauma. The amount of trauma required to give 50% mortality was directly related to the length of the rest interval. When this was 15 min., 2500 turns was followed by only 17% mortality.

Interrupted trauma was found to be followed by resistance after a latent period of 24 hr. The resistance was not as great as that produced by the same amount of continuous trauma.

Attempts to transfer resistance by transfusion of rat serum were not successful nor were other experiments designed to explain the mechanism of resistance.

The evidence presented suggests that some toxic substance is produced by trauma. This may either cause the death of the animal, or in 24 hr. lead to a resistance to subsequent trauma.

During the past three years extensive studies have been carried out on small animals subjected to graded degrees of trauma. In 1942, Noble and Collip (6) described a method of producing trauma in rats and guinea-pigs; the animals were rotated in a metal drum in such a manner that they received trauma from inside projections. By varying the number of turns, a curve of mortality related to the degree of trauma could be constructed. Traumatized animals that died without obvious complicating injuries such as haemorrhage were believed to have died from traumatic shock. It was believed that the results presented could best be explained on the theory that death following trauma was due to the liberation of a toxic substance from damaged tissue. The same method for the production of shock has been studied by Clarke and Cleghorn (3), Zahl, Hutner, and Cooper (9), and Chambers, Zweifach, and Lowenstein (1).

The development of a quantitative method for producing shock made it practical to determine the effects of trauma on adrenalectomized animals and to evaluate the results of treatment with adrenal cortical extracts (7). The increased susceptibility of adrenalectomized rats to trauma was found to be reduced by treatment with adrenal cortical extract but only slightly

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affected by desoxycorticosterone acetate. Both forms of treatment only slightly increased the tolerance of the normal rat to trauma. Similarly, pituitary corticotrophin produced little resistance.

In a previous paper (5) it was reported that all but one of 104 rats that were exposed to repeated doses of trauma were found to develop a resistance to amounts that were almost inevitably fatal to normal animals. Resistance in the rat was defined as its ability to withstand 1000 turns in the drum, whereas 800 turns caused approximately 100% mortality in the normal rat. The resistant state was found to be readily maintained even after months of repeated trauma and to persist for a prolonged interval after the trauma had ceased. Rats that were resistant to trauma were found not to develop the vascular engorgement of the intestines or the rupture of the viscera that may be found in normal traumatized animals. The resistant condition was still found to be present when the animals were traumatized under nembutal anaesthesia, so that any voluntary interference tending to lessen the degree of trauma was impossible. Normal guinea pigs and adrenalectomized rats could also be made resistant. The development of resistance to trauma has been confirmed by Chambers (2) and by Zahl *et al.* (9). It was believed that resistant animals would be of value in differentiating the toxic properties of various tissue extracts, since the toxin causing the death of a normal rat should not be effective in a resistant one. Similarly, the toxic substance should possess the property of protecting the normal rat against trauma. Extensive studies of this nature have been conducted and will be reported at a later date.

A comparative study of biochemical changes occurring in the blood and tissues of normal and trauma-resistant rats following trauma showed a marked difference between the two groups. In the normal rat, non-protein nitrogen, sugar, phosphate, lactic and pyruvic acid, were all markedly increased, some as much as 100%, while in the resistant animal the changes were negative or not significant (4).

In the present report investigations on the mechanism of the development of resistance to trauma are discussed. The minimal amount of trauma necessary to produce resistance has been determined and the necessity of a latent period in its production is stressed. Further evidence favouring the presence in shock of a toxic substance and experiments indicating its rate of destruction in the body and the subsequent development of resistance are given.

Methods

The apparatus for producing trauma was essentially that previously reported except that three pairs of drums were driven in series so that six animals could be run simultaneously. The drum speed was 40 r.p.m. There were two projections in each drum so that an animal received 80 impacts every minute. Hooded female rats of a strain maintained in the Institute and fed on Purina Fox Chow were used, and when strictly comparable results were required

animals of approximately the same age, weight, and sex were selected. Haemoglobins were measured in an Evelyn photoelectric colorimeter, 0.02 cc. of blood drawn from the tail being used.

Results

1. Resistance Following a Single Exposure to Continuous Trauma

In previous experiments it was shown that by gradually increasing the amounts of trauma over a period of 10 to 14 days rats could be made completely resistant to amounts of trauma inevitably fatal to the normal animals. It was also noted that some degree of resistance was afforded when rats were subjected to a run of 300 turns on two successive days. It was decided, therefore, to determine what amount of resistance could be established by a single exposure to trauma, how soon such resistance would develop, and how long it would last. Female rats of approximately 150 gm. therefore received a single run of either 300 or 400 turns in the drum. After intervals varying from three hours to 12 days they were subjected to 1000 turns and the number of animals that died was recorded. In control experiments on a group of 20 rats a mortality rate of 95% was found after they had received 1000 turns. The results on 203 animals are shown in Table I.

TABLE I
RESISTANCE FOLLOWING A SINGLE EXPOSURE TO TRAUMA

Interval between traumata	No. of rats	Mortality, % ¹	No. of rats	Mortality, % ²
Hours, 3	16	63	8	88
6	8	63	8	75
24	12	25	8	0
Days, 2	8	63	12	17
3	20	70	20	15
4	8	75	12	8
6	16	19	7	57
12	12	50	8	75
Controls	20	1000 turns only—95% mortality		

¹ Initial trauma = 300 turns; final trauma = 1000 turns.

² Initial trauma = 400 turns; final trauma = 1000 turns.

It may be seen that with an interval of three or six hours after the initial trauma of either 300 or 400 turns, the mortality following 1000 turns was only slightly (63 to 88%) less than in the control group (95%). When the initial trauma was 300 turns definite protection was found after an interval of 24 hr. After 2, 3, 4, and 12 days it was only slight, but the rats run at six days showed greater protection. Apparently the trauma caused by 300 turns was just enough to give demonstrable protection 24 hr. later. When, however, the initial trauma was 400 turns, the resistance was much more pronounced.

After one, two, three and four days a high degree of protection was present. This apparently had worn off after 6 to 12 days. Many of the animals that died showed evidence of partial protection since they survived for much longer periods after trauma than did normal rats. Resistance, therefore, followed a single exposure to trauma and was proportional to the degree of trauma. The resistance was not present six hours after trauma but had developed after a latent period of 24 hr.

Haemoglobin determinations were made on some of the rats after the initial and final traumata. After 300 or 400 turns little effect on haemoglobin was found except for some lowering. Animals that survived the final 1000 turns were seldom found to have increased hemoglobin values. Usually a progressive drop in the level was present indicating haemodilution. In contrast to this normal animals subjected to severe trauma showed increasing haemoglobin values over a period of six hours.

2. Mortality Following Exposure to Continuous Trauma

Since the previous papers on the mortality caused by this type of trauma a series of animals has been run, a slightly different technique being used. In this series, 123 large male rats, 275 to 350 gm. in weight, to be used for making extracts, were run in the drum until they were dead. The animals were inspected at every 100 turns after having received 800. After 1000 turns, 25% of the rats were dead, 52% after 1300, 77% after 1500, and 99% after 2000 turns. One animal died after 2300 turns. Rats that have been made resistant to 1000 turns in the usual fashion can readily stand increasing amounts of trauma, so that they can be made to tolerate 2400 turns of continuous trauma. This seems very striking in view of the control series of 123 animals, none of which survived this treatment.

3. Mortality Following Exposure to Interrupted Trauma

In a continuation of the experiments described in Section 1, the effect of reducing the intervals between traumata to a short time was observed. It was readily shown that if the period of trauma was interrupted for brief intervals the animals tolerated a greater number of turns than would have been expected if continuous trauma had been applied. In the following experiments, therefore, each period of trauma was that caused by 100 turns and the effect of rest intervals of from 1 to 20 min. between each 100 turns was determined. An attempt was made to find what amount of interrupted trauma under these conditions would cause approximately 50% mortality. The animals were not run until dead, as described in the previous section. When continuous trauma was applied to female control rats slightly less than 600 turns was followed by this per cent mortality. The effect of interrupted and continuous trauma on female rats is presented in Table II.

TABLE II
MORTALITY FOLLOWING INTERRUPTED TRAUMA

No. of rats	Interval between each 100 turns, min.	Total trauma—No. of turns	Mortality, %
101	Controls—None	300	1
86	Controls—None	400	12
26	Controls—None	600	58
12	Controls—None	800	100
6	1	100 × 6	33
6	1	100 × 7	17
6	1	100 × 9	66
6	2	100 × 11	50
6	2½	100 × 10	33
6	2½	100 × 11	83
8	5	100 × 10	0
8	5	100 × 15	50
6	10	100 × 24	66
6	15	100 × 25	17
6	20	100 × 18	0

From the above results it is obvious that interrupted trauma is less effective in causing mortality than continuous trauma. When intervals of one minute were allowed between each 100 turns it was found that 600 and 700 turns produced less than 50% but 900 turns caused 83% mortality. With an interval of two minutes one-half of the rats died after 1100 turns. When the interval was lengthened to two and one-half minutes 1000 to 1100 turns were required to kill 58% of the animals. Similarly, with five-minute intervals 1500 turns were needed. With 10-min. intervals 2400 turns caused 66% mortality and with 15-min. intervals 2500 turns killed 17% of the rats. With 20-min. intervals 1800 turns did not cause any mortality, the total period over which trauma was applied being in this case seven hours. If these results are plotted with the number of turns against intervals in minutes a direct linear relationship appears to be present. From this it is seen that with 20-min. intervals no mortality should result even if the experiment is continued for 24 hr. In this experiment it was noted that animals that died after trauma at 5, 10, and 15-min. intervals did not appear to show the vascular engorgement of the gastrointestinal tract that is commonly seen in the usual type of experiment. One explanation of these results might be that survival depends on the intervals, during which the body can destroy some toxic substance produced by trauma. Apparently this destruction takes place rapidly but is not complete even in an interval of 15 min. Since 83% of rats survived 2500 turns (an amount of continuous trauma that is inevitably fatal) these results also tend to support the evidence that mechanical damage *per se* is not a factor in mortality when trauma is produced by this method.

4. Effect of Interrupted Trauma Immediately Preceding Continuous Trauma

In the above experiments it was seen that when interrupted trauma was used, the rats were more resistant. It was possible, therefore, that under these conditions they might be developing resistance with great rapidity. This, however, seemed unlikely from the initial experiments described where a latent period up to 24 hr. after trauma appeared to be necessary before resistance became evident. To determine whether rats receiving interrupted trauma were developing resistance rapidly they were subsequently subjected to different amounts of continuous trauma. The results are tabulated in Table III.

TABLE III

EFFECT OF INTERRUPTED TRAUMA IMMEDIATELY PRECEDING CONTINUOUS TRAUMA

No. of rats	Initial trauma (interrupted)		Final trauma (continuous)	
	No. of turns	Intervals, min.	No. of turns	Mortality, %
86	Controls	None	400	12
26	Controls	None	600	58
12	Controls	None	800	100
12	100 × 4	5	400	17
12	100 × 4	5	700	58
6	100 × 4	5	1000	100
12	100 × 4	10	400	17
12	100 × 4	10	700	42
4	100 × 4	10	1000	100
2	100 × 18	20	1000	100

It may be seen that when rats received four series of 100 turns at five-minute intervals and were then subjected to 400, 700, or 1000 turns of interrupted trauma, they were no more resistant than normal rats. Similar results were obtained when 10-min. instead of five-minute intervals were used. Finally, two rats that had received 1800 turns at 20-min. intervals did not survive 1000 turns of continuous trauma. It seems clear, therefore, that the increased tolerance to interrupted trauma is a pseudo-resistance rather than a true one. Such a finding lends support to the view expressed above that the reduced effectiveness of interrupted trauma is due to the detoxification of some product during the rest periods.

5. Resistance Following Interrupted Trauma

Since interrupted trauma did not produce a true resistance immediately it was essential to determine whether this did develop after the usual latent period. The results of experiments of this type are shown in Table IV.

In the first part of the table either 400 or 800 turns were used to induce resistance and these were given with interruptions of five minutes, one hour,

TABLE IV

RESISTANCE FOLLOWING INTERRUPTED TRAUMA

No. of rats	Initial trauma (interrupted)		Time before final trauma	Final trauma (continuous)	
	No. of turns	Intervals		No. of turns	Mortality, %
10	100 × 4	5 min.	17 hours	1000	80
10	100 × 4	5 min.	2 days	1000	40
4	100 × 4	60 min.	2 days	1000	25
4	100 × 4	1 day	2 days	1000	50
6	100 × 8	5 min.	17 hours	1000	33
6	100 × 8	5 min.	2 days	1000	33
5	100 × 7	1 min.	2 days	1000	40
4	100 × 6	1 min.	4 days	1000	25
4	100 × 10	2½ or 5 min.	2 days	1000	0
3	100 × 10	2½ or 5 min.	5 days	1000	0
6	100 × 15	5 or 10 min.	2 days	1000	33
1	100 × 16	5 min.	5 days	1000	0

or 24 hr. Resistance was determined at 17 hr. or 48 hr. after the last trauma by subjecting the rat to 1000 continuous turns. Resistance was present in most rats so that it is apparent that after the usual latent period resistance is produced by interrupted trauma. Many of the animals listed in the preceding tables were also tested for resistance after intervals of two to five days. In all tests the initiating trauma was given in an interrupted form. These results are also shown in Table IV, and indicate that interrupted trauma is followed by the resistant state after a latent period of 24 to 48 hr. This lasts at least five days. If one compares the amount of resistance that develops after continuous trauma (Table I) with that after interrupted trauma, it is evident that for any given number of turns the continuous trauma is more effective.

6. Other Factors in Relation to Resistance

A number of varied experiments have been performed in attempts to elucidate the underlying mechanism of the resistant condition. Early trials were made to determine whether the serum of resistant rats would protect normal ones against death from traumatic shock, or whether the serum from rats that died following trauma would be toxic or would confer protection. Either fresh or lyophilized serum was given by subcutaneous, intraperitoneal, or intravenous injection, 1 or 48 hr. before trauma or immediately afterwards. In some trials the total amount of serum obtained from a traumatized animal

was injected into a normal one. The injected rats show no untoward effects. All these experiments, listed in Table V, have yielded essentially negative results.

TABLE V
EFFECT OF SERUM TREATMENT

No. of rats	Source of serum	Total amount of serum, cc.	Treatment and time relation to trauma	Mortality after 800 turns, %
8	Rats Normal	1½	Intraperitoneal—1 hr. before and immediately after	75
6	Normal	2 to 5	Intraperitoneal—48 hr. before	83
2	Normal	2½	Intravenous—48 hr. before	50
12	Traumatized	1½	Intraperitoneal—1 hr. before and immediately after	92
8	Traumatized	1½ to 5	Intraperitoneal—48 hr. before	87
2	Traumatized	2½	Intravenous—48 hr. before	100
2	Traumatized, bled 48 hr. later	2½	Intravenous—1 hr. before	50
2	Traumatized, bled 48 hr. later	2½	Intravenous—48 hr. before	100
4	Trauma resistant	5	Subcutaneous and intraperitoneal—2 hr. before	100
4	Trauma resistant	2½ to 5	Intraperitoneal—48 hr. before	100

From the data presented it is obvious that no explanation of the resistance has been found from experiments with the serum.

Various substances were injected into some animals to determine whether they would give rise to or interfere with the development of resistance. The results may be briefly summarized. Rats that received adequate doses of heparin* to prevent blood coagulation were not resistant to trauma. One hundred mg. of Witte's peptone injected intraperitoneally or 100 mg. of histamine dihydrochloride injected subcutaneously, 48 hr. prior to trauma, did not affect protection. The same dose of peptone did not interfere with the development of resistance after initial trauma of 400 turns.

Repeated bleedings in rats were made to determine whether they were followed by the development of resistance. The animals were bled 2%, 1%, and 1% of their body weight by heart puncture at three-hour intervals; following this treatment there was a progressive decline in haemoglobin for 24 hr. After 48 hr. some of the rats were transfused with citrated rat blood to raise the haemoglobin to nearly normal values. When these animals received 1000 turns they did not exhibit any degree of protection from the previous treatment. In some early experiments the effects of a different type of anoxia were determined. Rats that had been used in experiments by

* Kindly supplied by Connaught Laboratories, Toronto.

Dr. A. H. Neufeld and had been adapted to low oxygen tension over a prolonged period were subjected to trauma. These animals did not exhibit any appreciable resistance to trauma.

The possibility that rats become adapted to the motion factor of the drum has been suggested by Zahl, Hutner, and Cooper (9). In order to rule out this possibility rats were placed in metal tubes that were held rigid in the periphery of the drum. The revolving motion did not have any demonstrable effect on the rats nor did it afford them any protection when they were subsequently exposed to the usual type of trauma.

Two conditions have been noted that reduced the resistance of animals in which it had been previously established. As previously reported (5), during an outbreak of *Salmonella* infection the resistance of infected animals was lost even though their general condition appeared good. More recently some resistant rats were accidentally exposed for a few hours to moist heat sufficient to kill a number of animals. A number of those that survived were found during the next week to have lost their resistance to trauma.

Discussion

The results that have been described concern some of the factors that may influence the development of resistance to trauma in rats and the effects of interrupted trauma. It was found that appreciable degrees of resistance could be produced by a single exposure to small amounts of trauma, and that the degree of resistance was proportional to the amount of trauma. Whereas this might not be as marked or as complete as when repeated amounts of trauma were given over a period of time as a stimulus, the general resistance response appeared to be essentially the same. The resistance that developed after trauma did not become apparent immediately but a latent period of 24 to 48 hr. was essential for its production. The resistance after a single trauma might last longer than six days.

When rats were exposed to repeated small amounts of trauma separated by intervals it was found that this caused less mortality than when an equal amount of trauma was given continuously. The lengths of the intervals between runs of 100 turns appeared to be directly related to the total number of turns necessary to cause mortality, the longer the interval, the greater the amount of trauma required. With an interval of 20 min. no mortality was found. These results strongly suggested that some toxic product from damaged tissue was being detoxified in the body. This process was apparently rapid, but with intervals of less than 20 min. enough toxic substance appeared to accumulate so that after prolonged repeated trauma death would occur. Animals that tolerated this interrupted form of trauma did not immediately develop resistance, since if further continuous trauma was given they responded like normal animals. If a latent period of 24 to 48 hr. was allowed to elapse, however, after interrupted trauma, the animals were then resistant. It is believed that these results strongly support the toxin theory

of traumatic shock. Apparently the toxin is rapidly destroyed in the body, and this is followed in 24 to 48 hr. by a condition in which the animal is resistant to trauma. It is possible that the product of detoxification in some way produces the resistant condition in the animal.

Little information was obtained on the nature of the resistance to trauma. Attempts to transfer resistance by transfusion of rat serum proved ineffective. Whether serum was obtained immediately after trauma, or from fully resistant rats did not affect the results. Similarly, giving it immediately or 48 hr. before, or after, trauma did not give protection. In this type of experiment the rat, unlike the guinea-pig, as reported by Ungar (8), did not appear to be protected by serum from traumatized animals.

Other attempts to determine the mechanism of resistance have met with little success. The use of heparin to prevent blood clotting was not followed by resistance to trauma. The motion factor as suggested by Zahl, Hutner, and Cooper (9) was not important since the drum motion *per se* was not found to induce resistance to trauma. From the evidence described it seems most likely that the resistant condition is due to the effects of some substance liberated following trauma. Such a substance may be toxic and either cause death of the animal, or after 24 hr. impart a resistance to subsequent trauma. Experiments on this hypothesis are in progress.

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